Comparison of electropharmacological effects between terfenadine and its active derivative fexofenadine using a cross-over study in halothane-anesthetized dogs to analyze variability of pharmacodynamic and pharmacokinetic profiles of terfenadine and torsadogenic risk of fexofenadine

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ABSTRACT — In order to better understand the variability of pharmacodynamic and pharmacokinetic profiles of terfenadine between the previous studies as well as to qualitatively and quantitatively examine the proarrhythmic potential of its major active metabolite fexofenadine in comparison with that of terfenadine, we directly compared their electropharmacological effects with halothane-anesthetized dogs (n = 3). For this purpose, we adopted a cross-over design, which can directly compare the effects of terfenadine and fexofenadine under the identical metabolic condition. Terfenadine in doses of 0.03 and 0.3 mg/kg increased the mean blood pressure, but that of 3 mg/kg decreased it. Terfenadine also increased the heart rate and ventricular contractility in a dose-related manner; but delayed the atrioventricular nodal and intraventricular conductions as well as repolarization suggesting its proarrhythmic potential. Meanwhile, fexofenadine in the same dose increased the mean blood pressure in a dose-related manner without affecting any of the electrophysiological variables in the same animals that proarrhythmic risk of terfenadine was confirmed, indicating its lack of proarrhythmic risk. Peak plasma concentrations for fexofenadine were 3.7, 8.1 and 11.2 times greater than for terfenadine at each matching dose, indicating terfenadine may be metabolized much faster than fexofenadine. Taken together, after the low and middle doses of terfenadine, vasopressor effect of a metabolite fexofenadine could be greater than the depressor effect of parent compound terfenadine, but its reverse would be correct after the high dose. Thus, the cross-over analysis can be an effective way to better understand drug-induced cardiovascular responses.

Key words: Terfenadine, Fexofenadine, Cardiac electrophysiology, Cross-over study, Pharmacokinetic variability

INTRODUCTION

A second-generation antihistamine, terfenadine was withdrawn from the market in 1997 because of its high risk for QT-interval prolongation followed by the onset of torsade de pointes, i.e., drug-induced long QT syndrome (US Food and Drug Administration, 1997). Terfenadine has been reported to suppress $I_{Na}$, $I_{Ca,L}$ and $I_{Kr}$ in vitro.
its cardiovascular effects between the previous 2 evidently contradictory results have been reported regarding its macodynamic and pharmacokinetic profiles of terfenadine between the studies (Tashibu et al., 2005; Usui et al., 1998) as well as to qualitatively and quantitatively compare the effects of terfenadine and fexofenadine under the identical metabolic condition (Sugiyama, 2008).

MATERIALS AND METHODS

All experiments were approved by Toho University Animal Care and User Committee (No. 12-52-151) and the Toxicology Laboratory of Mitsubishi Tanabe Pharma Corporation (Test No. 4R908). Experiments were performed according to the Guidelines for care and use of laboratory animals of the Japanese Society of Toxicology and at the Toxicology Laboratory of Mitsubishi Tanabe Pharma Corporation. Experiments were performed at the Toxicology Laboratory of Mitsubishi Tanabe Pharma Corporation with 3 male beagle dogs obtained through Kitayama Labes Co., Ltd. (Nagano, Japan). They were kept in individual cages on a 12 hr light (7:00-19:00)-dark (19:00-7:00) cycle and their clinical signs were evaluated at least once daily during the experimental period.

Cardiohemodynamic parameters

The animals (n = 3) weighing 10.0-12.5 kg on experimental days were anesthetized initially with thioental sodium (30 mg/kg, i.v.). After intubation with a cuffed endotracheal tube, 1% halothane vaporized with 100% oxygen was inhaled with a volume-limited ventilator (SN-480-3, Shinano Manufacturing Co., Ltd., Tokyo, Japan). Tidal volume and respiratory rate were set at 20 mL/kg and 15 strokes/min, respectively. Four sets of clinically available catheter-sheath kit (FAST-CATH™ 406108; St. Jude Medical Daig Division, Inc., Minnetonka, MN, USA) were used; two were placed in the right and left femoral arteries, whereas the other two were done in the right and left femoral veins, respectively. To prevent blood clotting, heparin sodium (100 IU/kg) was intravenously administered through a flush line of the catheter sheath placed at the right femoral vein. A pig-tail catheter was placed at the left ventricle through the right femoral artery to measure the left ventricular pressure, whereas the aortic pressure was measured at a space between inside of the catheter sheath and outside of the pig-tail catheter through a flush line. The maximum upstroke velocity of the left ventricular pressure (LVdP/dtmax) and the left ventricular end-diastolic pressure were obtained during sinus rhythm to estimate the contractility and the preload to the left ventricle, respectively. A thermodilution catheter (SP5105L, Nihon
Kohden Corporation, Tokyo, Japan) was positioned at the right side of the heart through the right femoral vein. The cardiac output was measured with a standard thermodilution method by using a cardiac output computer (MTC-6210, Nihon Kohden Corporation). The total peripheral vascular resistance was calculated with the basic equation: total peripheral vascular resistance = mean blood pressure/cardiac output.

**Electrophysiological parameters**

The surface lead II electrocardiogram was obtained from the limb electrodes. A standard 6 French quad-polar electrodes catheter (Cordis-Webster Inc., Baldwin Park, CA, USA) was positioned at the non-coronary cusp of the aortic valve through the left femoral artery to obtain the His-bundle electrogram. A bi-directional steerable monophasic action potential recording/pacing combination catheter (1675P, EP Technologies, Sunnyvale, CA, USA) was positioned at the endocardium of the right ventricle through the left femoral vein to obtain monophasic action potential signals. The signals were amplified with a DC preamplifier (DAM50, World Precision Instruments, Sarasota, FL, USA). The duration of the monophasic action potential was measured as an interval, along a line which was horizontal to the diastolic baseline, from the monophasic action potential upstroke to the desired repolarization level. The interval (ms) at 90% repolarization level was defined as MAP$_{90}$. The right ventricle was electrically driven using a cardiac stimulator (BC-03, Fukuda Denshi Co., Ltd., Tokyo, Japan) with the pacing electrodes of the monophasic action potential recording/pacing combination catheter. The stimulation pulses were rectangular in shape, 1-2 V (about twice the threshold voltage) and 1-ms duration. The MAP$_{90}$ was measured during sinus rhythm (MAP$_{90\,\text{(sinus)}}$) and at a pacing cycle length of 400 ms (MAP$_{90\,\text{(CL400)}}$) and 300 ms (MAP$_{90\,\text{(CL300)}}$). The effective refractory period of the right ventricle was measured by the programmed electrical stimulation. The pacing protocol consisted of 5 beats of basal stimuli in a cycle length of 400 ms followed by an extra stimulus of various coupling intervals. Starting in late diastole, the coupling interval was shortened in 5 ms decrements until refactoriness occurred. The duration of the terminal repolarization period of the ventricle was calculated by the difference between the MAP$_{90\,\text{(CL400)}}$ and effective refractory period at the same site, which reflects the extent of electrical vulnerability of the ventricular muscle (Sugiyama, 2008).

**Experimental protocol**

The study was conducted with a cross-over design under sterile condition. Terfenadine was initially given to 2 dogs, whereas the other dog initially received fexofenadine. Benzylpenicillin potassium and streptomycin sulfate were intramuscularly given to the animals on the day and the next day following each experiment. About 2 months later, the other drug was given to the animals. In each experiment, the aortic pressure, left ventricular pressure, electrocardiogram, His-bundle electrogram and monophasic action potential signals were continuously monitored with a polygraph system (RM-6000, Nihon Kohden Corporation), and analyzed with a real-time fully automatic data analysis system (MP/ VAS3 for Windows ver 1.6, Physio-Tech Co., Ltd., Tokyo, Japan). Each measurement of electrophysiological and monophasic action potential variables as well as atrio-His and His-ventricular interval was made based on the mean of 3 consecutive recordings of consecutive complexes. The cardiovascular variables were assessed in the following order. The electrocardiogram, His-bundle electrogram, systemic and left ventricular pressure, and monophasic action potential signals were recorded during sinus rhythm. Next, the cardiac output was measured three times. Then, monophasic action potential signals were recorded during the ventricular pacing at a cycle length of 400 and 300 ms. Finally, the ventricular effective refractory period was measured at a cycle length of 400 ms. All variables described above were usually obtained within 1 min at each time point.

After the basal assessment, terfenadine or fexofenadine in a low dose of 0.03 mg/kg was intravenously administered over 10 min and each variable was assessed 5, 10, 15, 20 and 30 min after the start of the infusion. Then, the drug in a middle dose of 0.3 mg/kg was intravenously administered over 10 min and each variable was recorded in the same manner. Finally, the drug in a high dose of 3 mg/kg was intravenously administered over 10 min and each variable was assessed 5, 10, 15, 20, 30, 45 and 60 min after dosing.

**Measurement of plasma drug concentrations**

A volume of 3 mL of blood was drawn from the left femoral artery to measure the plasma drug concentration at 5, 10, 20 and 30 min after the start of the low and middle doses and at 5, 10, 20, 30 and 60 min after the high dose. The blood samples were centrifuged at 1,500 × g for 30 min at 4°C. The supernatant plasma was stored at −80°C until the drug concentration was measured. The plasma concentrations of terfenadine and fexofenadine were determined by HPLC (LC10A series, Shimadzu Corporation, Kyoto, Japan) followed by MS/MS (API 4000, Applied Biosystems/MDS SCIEX, Foster, CA, USA). The detection limit was 1 ng/mL for terfen
dine and fexofenadine.

Drugs
Terfenadine and fexofenadine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Each drug was dissolved in 1% lactate solution in a concentration of 3 mg/mL, which was further diluted to 0.3 and 0.03 mg/mL with 1% lactate solution, respectively. The following drugs were purchased: thiopental sodium (Mitsubishi Tanabe Pharma Corporation, Osaka, Japan), halothane (Takeda Chemical Industries, Osaka, Japan), heparin sodium (Mitsubishi Tanabe Pharma Corporation), benzylpenicillin potassium (Meiji Seika Pharma Co., Ltd., Tokyo, Japan) and streptomycin sulfate (Meiji Seika Pharma Co., Ltd.).

Statistical analysis
Data are presented as mean ± S.E. The pre-drug control values between the terfenadine and fexofenadine groups were compared with a paired $t$-test. The statistical significances within a parameter were evaluated by one-way repeated-measures analysis of variance (ANOVA) followed by Contrasts as a post-hoc test for mean values comparison, whereas those between the extents of the prolongation in the MAP$_{90}$ and MAP$_{90(LC100)}$ were assessed by two-way repeated-measures ANOVA. A $p$-value < 0.05 was considered to be significant.

RESULTS
No animals exerted any lethal ventricular arrhythmias or hemodynamic collapse, leading to the animals’ death during the experiment. No significant difference was detected in any of the pre-drug control values between the terfenadine and fexofenadine.

Plasma drug concentration
The time courses of the plasma concentration of terfenadine and fexofenadine are summarized in Fig. 1A (n = 3 for each group). The peak plasma concentrations of terfenadine after 0.03, 0.3 and 3 mg/kg of infusion were 68 ± 29, 400 ± 56 and 1,930 ± 485 ng/mL, respectively. The plasma concentration of terfenadine 60 min after the administration of the high dose was 263 ± 71 ng/mL. The peak plasma concentrations of fexofenadine after the administration of the low, middle and high doses were 252 ± 29, 3,250 ± 747 and 21,645 ± 956 ng/mL, respectively. Thus, the peak plasma concentrations were 3.7, 8.1 and 11.2 times greater for fexofenadine than for terfenadine at each matching dose, demonstrating that terfenadine can be metabolized much faster than fexofenadine.

Effects on the cardiohemodynamic variables
The time courses of changes in the heart rate, mean blood pressure, cardiac output, LVdP/dt$_{max}$, left ventricular end-diastolic pressure and total peripheral vascular resistance are summarized in Figs. 1A and 1B (n = 3 for each group). Their pre-drug control values (C) were 89 ± 10 beats/min, 112 ± 7 mmHg, 1.7 ± 0.1 L/min, 1,849 ± 314 mmHg/s, 15 ± 3 mmHg and 65.9 ± 1.6 mmHg (L/min)$^2$ for terfenadine, whereas those were 90 ± 7 beats/min, 110 ± 3 mmHg, 1.8 ± 0.2 L/min, 1,832 ± 253 mmHg/s, 14 ± 3 mmHg and 63.8 ± 4.5 mmHg (L/min)$^2$ for fexofenadine, respectively. Administration of 0.03 mg/kg of terfenadine increased the mean blood pressure for 15-30 min, whereas no significant change was detected in the other variables. Additional administration of 0.3 mg/kg of terfenadine increased the heart rate at 10 min, mean blood pressure for 15-30 min and cardiac output for 5-15 min, but decreased the total peripheral vascular resistance for 5-10 min, whereas no significant change was detected in the other variables. Additional administration of 3 mg/kg of terfenadine increased the heart rate for 5-20 min, cardiac output for 5-20 min and LVdP/dt$_{max}$ for 5-15 min, but decreased the mean blood pressure for 5-10 min, left ventricular end-diastolic pressure for 5-10 min and total peripheral vascular resistance for 5-20 min. Then, the total peripheral vascular resistance increased at 60 min. On the other hand, administration of 0.03, 0.3 and 3 mg/kg of fexofenadine increased the mean blood pressure in a dose-related manner, and significant change was detected at 10 and 20 min, for 5-30 min, and 5-60 min after the respective doses, whereas no significant change was detected in the other variables.

Effects on the electrocardiogram during the sinus rhythm
Typical tracings showing the effects of terfenadine and fexofenadine on the electrocardiogram are depicted in Fig. 2, whereas the time courses of changes in the PR interval, QRS width and QT interval are summarized in Fig. 3A. Their pre-drug control values (C) were 123 ± 13, 53 ± 1 and 294 ± 13 ms for terfenadine, whereas those were 124 ± 13, 56 ± 4 and 289 ± 13 ms for fexofenadine, respectively. Administration of 0.03 mg/kg of terfenadine increased QRS width at 30 min, whereas no significant change was detected in the PR or QT interval. Additional dosing of 0.3 mg/kg of terfenadine increased QRS width at 30 min, whereas no significant change was detected in the PR or QT interval. Additional dosing of 0.3 mg/kg of terfenadine did not alter these variables. Three mg/kg of terfenadine prolonged the PR interval at 45 min and QRS width for 5-60 min, whereas it shortened the QT interval for 5-10 min, but prolonged it for 30-60 min.
Fig. 1. A. Time courses of the plasma concentration, heart rate (HR) and mean blood pressure (MBP) of terfenadine (left panels) and fexofenadine (right panels). B. Time courses of the cardiac output (CO), maximum upstroke velocity of left ventricular pressure (LVdP/dtmax), left ventricular end-diastolic pressure (LVEDP) and total peripheral vascular resistance (TPR) of terfenadine (left panels) and fexofenadine (right panels). Data are presented as mean ± S.E. (n = 3). The closed symbols represent statistical change from each pre-drug control (C) value by p < 0.05.
On the other hand, no significant change was detected in any of the variables by fexofenadine for any dose.

Effects on the His bundle electrogram and MAP\textsubscript{90} during sinus rhythm

The time courses of changes in His bundle electrogram and monophasic action potential are summarized in Fig. 3A. The pre-drug control values (C) of the atrio-His and His-ventricular intervals, and MAP\textsubscript{90 CL300}, were 95 ± 13, 29 ± 3 and 238 ± 10 ms for terfenadine, whereas those were 100 ± 8, 26 ± 2 and 232 ± 15 ms for fexofenadine, respectively. Administration of 0.03 mg/kg of terfenadine did not change these variables. Additional dosing of 0.3 mg/kg of terfenadine increased the MAP\textsubscript{90 CL300} for 15-30 min, whereas no significant change was detected in the other variables. Further additional administration of 3 mg/kg of terfenadine prolonged the atrio-His interval for 20-60 min and MAP\textsubscript{90 CL300} for 15-60 min, whereas no significant change was detected in the His-ventricular interval. On the other hand, no significant change was detected in any of the variables by fexofenadine for any of the doses.

Effects on the MAP duration during the ventricular pacing, effective refractory period and terminal repolarization period

The time courses of changes in the MAP\textsubscript{90 CL300}, MAP\textsubscript{90 CL400}, effective refractory period and terminal repolarization period are summarized in Fig. 3B. Their pre-drug control values (C) were 220 ± 6, 237 ± 7, 205 ± 6 and 32 ± 3 ms for terfenadine, whereas those were 210 ± 8, 230 ± 12, 208 ± 14 and 22 ± 4 ms for fexofenadine, respectively. Administration of 0.03 mg/kg of terfenadine did not change these variables. Additional dosing of 0.3 mg/kg of terfenadine prolonged the MAP\textsubscript{90 CL300} for 15-30 min, MAP\textsubscript{90 CL400} for 5-30 min and effective refractory period for 5-30 min, whereas no significant change was detected in the terminal repolarization period. Further additional administration of 3 mg/kg of terfenadine prolonged the MAP\textsubscript{90 CL300} for 15-60 min, MAP\textsubscript{90 CL400} for...
Fig. 3. A. The time courses of the PR interval (triangles), QRS width (circles), QT interval (squares), atrio-His interval (AH; circles), His-ventricular interval (HV; triangles) and duration of monophasic action potential at 90% repolarization level (MAP$_{90\text{sinus}}$; squares) during sinus rhythm of terfenadine (left panels) and fexofenadine (right panels). B. Time courses of the MAP$_{90}$ at a pacing cycle length of 300 ms (MAP$_{90\text{CL300}}$; triangles) and 400 ms (MAP$_{90\text{CL400}}$; circles); effective refractory period at a basic pacing cycle length of 400 ms (ERP); and terminal repolarization period (TRP = MAP$_{90\text{CL400}}$ – ERP) of terfenadine (left panels) and fexofenadine (right panels). Data are presented as mean ± S.E. (n = 3). The closed symbols represent statistical change from each pre-drug control (C) value by p < 0.05.
15-60 min and effective refractory period for 10-60 min, whereas it shortened the terminal repolarization period for 5-15 min, but prolonged it for 30-60 min. The extent of prolongation of the MAP_{90(CL400)} was significantly greater than that of the MAP_{90(CL300)} indicating characteristics of reverse-frequency dependent prolongation of repolarization by terfenadine. On the other hand, no significant change was detected in any of the variables by fexofenadine for any of the doses.

**DISCUSSION**

In the present study, we investigated the cardiovascular effects and pharmacokinetic profiles of terfenadine and fexofenadine by using a cross-over study in the halothane-anesthetized dogs (Ishizaka et al., 2010; Izumi-Nakaseko et al., 2014; Sugiyama et al., 2001; Sugiyama, 2008; Takahara et al., 2005; Usui et al., 1998). While terfenadine showed significant cardiohemodynamic and electrophysiological effects, fexofenadine did not alter them except for an increase in the mean blood pressure.

**Cross-over design**

We adopted a cross-over design in 3 halothane-anesthetized dogs. No significant difference was detected in any of the pre-drug control values between the terfenadine and fexofenadine, which were obtained from the same animal group, confirming the stability and reproducibility of currently used experimental system in vivo. Also, it should be noted that the current study design could halve the total number of the animals while improving the assay sensitivity and reliability, which may partly contribute to animal welfare. Thus, the use of cross-over design may become an effective way for animal studies to compare the pharmacodynamic profile of multiple compounds under the same pharmacokinetic conditions, as it has already been adopted for clinical pharmacology studies.

**Rationale for dose selection**

Therapeutically recommended oral dose of terfenadine was 60 mg twice daily, and its peak plasma concentration after oral 60 mg twice daily in humans has been reported to be 6.4 ± 6.2 ng/mL (Abernethy et al., 2001). Therefore, the peak plasma concentrations of terfenadine ranging from 68 to 1,930 ng/mL in this study can be supratherapeutic. On the other hand, therapeutically recommended oral dose of fexofenadine was 60 mg twice daily or 180 mg once daily (Pratt et al., 1999; Russell et al., 1998), and its peak plasma concentrations after oral 40 mg twice daily and oral 180 mg once daily in humans have been reported to be 220 and 625 ng/mL, respectively (Mendoza et al., 2007; Russell et al., 1998). Thus, the peak plasma concentrations of fexofenadine ranging from 252 to 21,645 ng/mL in this study can be clinically relevant to supratherapeutic. It should be also noted that peak plasma concentrations of fexofenadine were 3.7-11.2 times greater than those of terfenadine, although the same doses were intravenously given to the same animals in this study. These results together with previous information confirm that terfenadine may be metabolized much faster than fexofenadine after intravenous administration in dogs (Leeson et al., 1982).

**Cardiohemodynamic effects**

Fexofenadine significantly increased the mean blood pressure in a dose-related manner, whereas it did not alter the other cardiohemodynamic variables, although fexofenadine was described to induce hypertensive response only in < 0.1% of patients according to the interview form from the manufacturer. The mechanisms of fexofenadine-induced hypertension need to be further elucidated. Meanwhile, the low and middle doses of terfenadine gradually increased the mean blood pressure, but the high dose immediately and transiently decreased it. Since plasma protein binding rate of terfenadine is 97% (Redfern et al., 2003), the peak unbound plasma concentrations can be calculated to be 2, 12 and 58 ng/mL after the administration of the low, middle and high doses, respectively. Terfenadine unlike fexofenadine has been reported to inhibit the IC_{50} in the human atrial myocytes with IC_{50} of 185 nM (87.3 ng/mL) (Hove-Madsen et al., 2006), which may provide the rationale for the currently observed vasodilator actions after the middle and high doses of terfenadine. In addition, terfenadine has been reported to be largely metabolized into fexofenadine (Leeson et al., 1982; Lalonde et al., 1996). Thus, the temporal pattern of change in the mean blood pressure may depend on the net balance between depressor action of terfenadine by itself and vasopressor effect of its major metabolite fexofenadine; namely, vasopressor effect of a metabolite fexofenadine could be greater than the depressor effect of parent compound terfenadine after the low and middle doses, but its reverse would be correct after the high dose.

Maximum plasma concentrations of terfenadine after intravenous infusion of 3 mg/kg over 10 min have been reported to be 15,390 ng/mL by Usui et al. (1998) in the halothane-anesthetized beagle dogs and 1,177 ng/mL by Tashibu et al. (2005) in the isoflurane-anesthetized beagle dogs, which was 1,930 ng/mL in this study. These results indicate that large pharmacokinetic variability among the
studies may depend on the animal group rather than the anesthetics used, and can partly explain the differences of its cardiovascular effects in vivo (Tashibu et al., 2005; Usui et al., 1998). Namely, the most potent cardiovascular suppressive effects were reported by Usui et al. (1998), whereas the least ones were confirmed by Tashibu et al. (2005), extent of which were proportional to the parent compound terfenadine concentration. Similar pharmacokinetic variability was noticed in healthy human subjects (Lalonde et al., 1996).

On the other hand, terfenadine increased the heart rate, cardiac output and ventricular contraction in a dose-related manner, which would be induced by reflex-mediated increase of sympathetic tone resulting from its vasodilator and/or hypotensive actions via the inhibition of the \( I_{\text{ca,L}} \) (Hove-Madsen et al., 2006). Indeed, similar results of dose-related cardiohemodynamic responses were confirmed by dihydropyridine L-type Ca\(^{2+}\) channel blocker amlodipine in our previous study using the same halothane-anesthetized canine model (Ishizaka et al., 2010).

**Electrophysiological effects**

The high dose of terfenadine slowed the atrioventricular nodal conduction, reflecting an inhibition of \( I_{\text{ca,L}} \) in vivo as discussed above (Hove-Madsen et al., 2006). Importantly, the increase of sympathetic tone induced by the hypotensive action of terfenadine might have partly counteracted its atrioventricular nodal conduction delay, partly explaining its slow onset of the negative dromotropic effect. On the other hand, terfenadine prolonged the QRS width after the low and high doses without altering it after the middle dose, whereas no significant change was detected in the HV interval during the study. A previous in vitro study using the canine atrial cardiomyocytes indicated that terfenadine inhibited \( I_{\text{Na}} \) with IC\(_{50}\) of 930 nM (439 ng/mL) (Lu and Wang, 1999), and the peak unbound plasma concentrations in this study could be 58 ng/mL even after the high dose. Although the inhibition of \( I_{\text{Na}} \) may partly explain the prolongation of the QRS width after the high dose (Sugiyama et al., 1994), other factors need to be explored to fully understand terfenadine-induced change in the QRS width.

Terfenadine prolonged the repolarization phase in a dose-related manner; moreover, reverse use-dependent delay of repolarization was confirmed, suggesting that terfenadine may inhibit \( I_{\text{Kr}} \) in vivo. The hypothesis is supported by a previous in vitro study using HEK-293 cell that terfenadine suppressed \( I_{\text{Kr}} \) with IC\(_{50}\) of 56 nM (26.4 ng/mL) (Rampe et al., 1997). On the other hand, in a previous study using *Xenopus leavis* oocytes terfenadine decreased \( I_{\text{Kr}} \) by 4.5 % at 1 \( \mu \text{M} \) (471 ng/mL) (Siebrands et al., 2006), suggesting that the high dose of terfenadine may hardly inhibit \( I_{\text{Kr}} \). Rather, the high dose of terfenadine shortened the repolarization period for 5-10 min, indicating that reflex-mediated indirect \( I_{\text{Kr}} \) enhancement was greater than direct \( I_{\text{Kr}} \) suppression.

While the atrioventricular nodal and intraventricular conduction remained suppressed, repolarization delay was gradually enhanced with time after the high dose of terfenadine. Its unbound plasma concentration can be calculated to be 7.9 ± 2.1 ng/mL at 60 min after the high dose, which was 55.6, 11.5 and 3.3 times smaller than the IC\(_{50}\) values of \( I_{\text{Na}} \), \( I_{\text{ca,L}} \) and \( I_{\text{Kr}} \), respectively. The discrepancy of the results between the pharmacokinetics and pharmacodynamics suggests that terfenadine might be accumulated in the cardiomyocytes, which might be partly supported by its high lipophilicity (logD\(_{oct} = 4.4\) (Siebrands et al., 2006).

Fexofenadine did not affect any of the electrophysiological variables in the same animals that electrophysiological effects of terfenadine were demonstrated, indicating that fexofenadine by itself lacks electrophysiological activity on the heart in vivo. This conclusion is in good accordance with the previous in vitro results that fexofenadine did not suppress \( I_{\text{ca,L}} \) in human atrial myocytes (Hove-Madsen et al., 2006), \( I_{\text{Na}} \) in *Xenopus leavis* oocytes (Roy et al., 1996) or \( I_{\text{Kr}} \) (interview form from the manufacturer).

**Proarrhythmic potentials**

The drug-induced prolongation of terminal repolarization period has been shown to be a reliable marker that can predict the onset of slow conduction and re-entry, leading to perpetuation of torsade de pointes (Sugiyama, 2008). In this study, the high dose of terfenadine prolonged the terminal repolarization period for 30-60 min, indicating that terfenadine could provide such proarrhythmic potential. On the other hand, fexofenadine did not alter the terminal repolarization period even at the supratherapeutic concentrations in the same animals that proarrhythmic risk of terfenadine was confirmed, indicating that fexofenadine lacks proarrhythmic potential.

In conclusions, cross-over analysis of cardiovascular effects of terfenadine and fexofenadine may provide a clue to better understand previously reported unique pharmacodynamic profile of terfenadine. Fexofenadine may increase the blood pressure, but it will not alter any of the electrophysiological variables including the terminal repolarization period even at the supratherapeutic concentrations, indicating that fexofenadine lacks proarrhythmic potential.
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Conflict of interest---- HH, HM, NI and KT were employees of Mitsubishi Tanabe Pharma Corporation. There was no financial relationship with any organizations that might have an interest in the submitted work in the previous 5 years.

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